Agarose Gel Electrophoresis Protocol for ***RNA***

Reagents and Materials:

for preparation:
- tank, tray, comb
- Diethylpyrocarbonate (from Sigma, cat. nr. D-5758)
- 0.1% DEPC (Diethylpyrocarbonate) H_2O: mix 1 ml DEPC in 1000 ml H_2O and autoclave.
- normal melting agarose powder,
- 10 x TBE buffer solution, gel stain (Eco Safe Nucleic Acid Staining Solution)
- microwave, Erlenmeyer flask, measuring cylinder, scales

for loading: pipette, PCR tubes or tinfoil, power supply

for documentation: gel documentation system

Gel preparation:

1. - Prepare sufficient 1 x TBE electrophoresis buffer (1:10 dilution of TBE:DEPC H_2O)
   - Clean all tools using DEPC H_2O.
   - Position the comb 0.5-1 mm above the plate so that a complete well is formed when the agarose is added.
2. - Prepare agarose gel for a **1.2% agarose gel**:

   1.2 g agarose / 100 ml 1 x TBE buffer in Erlenmeyer flask

   - Cover the flask with kimwipes/ parafilm and heat with microwave until the agarose dissolves. Measure it again and complete the evaporated liquid with distilled water.
   - Leave it to cool down to about 60 °C on the bench for several minutes but do not leave it too long so the agarose should not start to solidify.
   - Stain the agarose solution:

     5 µl ECO Safe Nucleic Acid Staining Solution / 100 ml gel

   - Mix the agarose solution well by swirling the flask. Pour the agarose into the mold. (3-5 mm thickness)
3. - After 30 minutes at room temperature carefully remove the comb.
   - Position the gel into the gel electrophoresis tank. Avoid bubbles!

- Add enough TBE buffer to cover the gel to a depth of about 5 mm.

Loading:

1. Mix the samples of RNA with gel-loading buffer with pipettes:
   - 0,5 µg RNA
   - 2 µl loading dye

   to 10 µl RNase – free water
2. Load the mixtures slowly into the slots. Avoid making bubbles!
3. Attach the electrical leads so that RNA can move toward the anode. (Red lead)
4. Apply a voltage of 1-5 V/cm.
5. Run the gel until the gel-loading buffer stain have migrated the appropriate distance (normally until the bromophenol blue dye front migrated ¾ of the way down the gel).

Documentation:

1. Turn off the current and remove leads.
2. Examine the gels: Carefully put it on an ultraviolet transillumunator and take a photo.

Technical appendix:

- Type of Agarose: normal melting point, molecular grade
- Agarose solution: The excess solution can be stored at 4 °C until later use.
- 10 x TBE solution (per liter): from molecular grade reagents
  + 108 g Tris
  + 55.65 g boric acid
  + 40 ml 0.5M EDTA (pH 8.0)

  stored at room temperature
- Gel stain: Eco Safe Nucleic Acid Staining Solution (Pacific Image Electronics)
- Loading dye (6X concentration):

  0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in water
  store at 4 °C